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Osteoarticular infections in paediatric sickle cell disease: in the era of multidrugresistant bacteria

Osteomyelitis (OM) is a major cause of morbidity in sickle cell disease (SCD) patients (Thanni, 2006; Tarer *et al.*, 2006) with *Staphylococcus aureus* and the *Salmonella* species being the most frequently isolated microorganisms (Burnett *et al.*, 1998; Thanni, 2006). In recent years, we have observed an increasing number of patients in our institution with severe multifocal, multidrug-resistant (MDR) osteoarticular infections (OAI). We are trying to determine OAI incidence, aetiology and management in children with SCD and evaluate MDR strain emergence.

We performed a longitudinal, prospective, observational data analysis of SCD children with OAI (<18 years old) admitted to a tertiary care paediatric hospital in Lisbon, Portugal from January 2010 to December 2018. Clinical and microbiological data, imaging, treatment and evolution were obtained. OM and septic arthritis (SA), complications and sequelae (Arnold & Bradley, 2001), and MDR were defined according to previous published criteria (Magiorakos *et al.*, 2012). Quantitative and categorical variables were compared using non-parametric tests. SPSS Statistics® version 24 was used.

Fifteen of the 165 (9·1%) patients with SCD had OAI, all SS homozygous, with a fetal hemoglobin level of 8·4 ± 6·6 (similar to other SCD patients), a median age of eight years [IQR2–11], 53·3% female, and all of African origin: nine lived in Portugal and six lived in Portuguese speaking african countries (PALOP) and travelled to Portugal for medical care.

The most frequent clinical and imaging findings are presented in Table I. The majority (40%) were admitted with the diagnosis of vaso-occlusive crisis. All were diagnosed with OM and 10 had concomitant SA. Five already had chronic OM on admission, and six had multifocal involvement (range 2–11). A pathogen was identified in seven patients (41%) (with one patient with SAMS plus *Pseudomonas*), by

Table I. Clinical and imagiologic data for 15 patients.

Variable	Value
Gap between symptoms and admission, days (median, IQR)	7 [5–22]
Gap between admission and diagnosis, median (median, IQR)	5 [2-12]
Pain (N, %)	15 (100)
Functional limitation (N, %)	13 (86.7)
Fever >38·2ºC (N, %)	11 (73.3)
Local inflammatory signs (N, %)	11 (73.4)
Leukocyte count >15·000/ml (N, %)	12 (80)
C-reactive protein >20 mg/ (N, %)	13 (86.6)
Sedimentation rate >20 mm/h (N, %)	15 (100)
Bones more commonly affected	
Humerus, N	6
Radius, N	5
Femur, N	4
Tibia, N	3
Joints more commonly affected	
Elbow, N	6
Shoulder, N	4
Hip, N	3
Radiographs	
Abnormal at admission (N, %)	11 (73·3)
Evident avascular necrosis (N, %)	6 (40)
Lytic changes (N, %)	7 (46·7)
Periosteal reaction(N, %)	8 (53·3)
Ultrasonography	
Abscesses (N, %)	6 (40)
MRI	
Abnormal (N, %)	15 (100)
Long bones osteonecrosis (N, %)	14 (93·3)
Signal intensity alterations and gadolinium (N, %)	15 (100)
Soft tissue abscesses (N, %)	10 (66·7)

positive cultures ($N = 8$) or molecular amplification ($N = 2$): blood culture in two (2/15; 13%), culture from synovial fluid in four (4/6; 67%) and bone sample in two (2/8; 25%). The most common identified bacteria were MDR *Serratia marcescens* ($N = 3$), methicillin-susceptible *S. aureus* ($N = 2$), MDR *Enterobacter cloacae* ($N = 1$), MDR *Pseudomonas aeruginosa* ($N = 1$) and *Enterococcus faecium* ($N = 1$; molecular amplification). Most patients were empirically treated with a third generation cephalosporin ($N = 8$) or flucloxacillin ($N = 4$) plus gentamicin. After bacterial identification, treatment was modified to meropenem ($N = 5$) plus amikacin ($N = 3$) or flucloxacillin ($N = 1$). The median duration of intravenous and total antibiotic treatment was 21 days (IQR 16–69) and six weeks (IQR 5–10 weeks), respectively. Ten patients were discharged on oral antibiotics: cefuroxime ($N = 5$), levofloxacin plus rifampicin ($N = 3$) or flucloxacillin ($N = 2$).

Eight (53%) patients underwent surgical procedures, seven more than one intervention (range 2–11), including arthrocentesis, arthrotomy, bone drainage and debridement, and 26·6% received hyperbaric oxygen therapy.

Table II. Comparing children with MDR bacteria with children with no isolation or susceptible bacteria.

Variable	MDR bacteria $N = 6$	No isolation or susceptible bacteria $N = 9$	P
Age (median, IQR, years)	10 [7–15.2]	5 [1·6–9.5]	0·04
Sex male (N, %)	1(16·7)	6 (6·7)	0·119
Previous multiple antibiotherapy (N, %)	6 (100)	0 (0)	0·000
Previous hospital admission (N, %)	6 (100)	6 (67)	0·2
PALOP provenance (N, %)	6 (100)	3 (33)	0·028
Days of symptoms to admission (Median, IQR, days)	22[9–105]	5 [1–8]	0·119
Fever (N, %)	5 (83)	6 (66)	0·6
CRP at admission (median, IQR, mg/l)	143 ± 58	77 ± 92	0·04
ESR at admission (median, IQR, mm/h)	115 ± 41·7	52 ± 25	0·04
Days of hospitalisation (median, IQR)	79·5 ± 94·7	20 ± 15	0·001
Days IV antibiotic (median, IQR)	70 ± 57	18 ± 15	0·04
Total weeks of antibiotics (median, IQR)	10 ± 8·4	6 ± 1·4	0·3
Number of Surgical interventions (median, IQR)	2·5 [2–5.8]	0·0 [0–0·5]	0·001
Complications (N, %)	6(100)	2 (22)	0·015
Sequelae at 12 months (N, %)	83	0	0·002

PALOP, Portuguese speaking african countries.

Comparing data from children with MDR bacteria ($N = 6$) to that of children with no isolation or susceptible bacteria ($N = 9$) (Table II), the median number of complications was three [IQR 2·5–5] *versus* 0 [IQR 0–0.5]. Complications in the MDR group included pathologic fractures ($N = 2$), intraosseous or subperiosteal abscesses ($N = 6$), avascular necrosis ($N = 4$) and progression to chronic osteomyelitis ($N = 5$). In the non-MDR group, complications were intraosseous abscesses ($N = 2$) and chronic osteomyelitis ($N = 1$). At 12 months follow-up, only the MDR subgroup presented sequelae: functional limitation ($N = 5$), dysmetria ($N = 2$), stiffness ($N = 1$) and angular deformity

($N = 1$). Two patients in the MDR group required a dorso-lumbo-sacral orthosis, three needed crutches and five needed physical therapy. Risk factors for MDR infections were provenance from PALOP (odds ratio [OR], 20·4], 95% confidence interval [CI], 0·855–489]) and previous multiple antibiotic courses (OR 247 [4·3–14 106]).

The OM incidence in our series (13%) was comparable to the 12–16·3% reported by Neonato *et al.* in 299 homozygous SCD patients in France (Neonato *et al.*, 2000), most Hb SS (Bennett & Namnyak, 1990), with a median age between nine and 15·7 years (Epps *et al.*, 1991; Sadat-Ali, 1998). We observed a higher rate of multifocal disease (41%) than usually described (10–34%) (Sadat-Ali, 1998; Chambers *et al.*, 2000), but similar to that reported by Ebong (42%) in Nigeria (Ebong,1986). These differences can most likely be explained by the small sample size of most series.

OM and osteonecrosis are often associated and coincident, varying from 49% to 74%, slightly higher than our data (40%). (Bennett & Namnyak, 1990; Hernigou *et al.*, 2010). Differentiating a bone infarct from OM is extremely difficult. As previously reported, in our study the combination of fever and local swelling, in an ill-appearing patient, together with a high CRP (C-reactive protein level) and ESR (erythrocyte sedimentation rate) and contrast-enhanced T1-weighted images was suggestive of infection (Chambers *et al.*, 2000; Almeida & Roberts, 2005; Hernigou *et al.*, 2010; Kosaraju *et al.*, 2017). Bone culture was only collected from complicated cases, justifying the low (40%) microbiologic yield in our study.

The aetiology is changing and varies geographically (Thanni, 2006). In most studies, *Salmonella* and *S. aureus* predominate, with a 2·2:1 ratio (Burnett *et al.*, 1998). However, other Gram-negative bacilli (GNB) (18–19·6%) ranked almost equal to *S. aureus* (Burnett *et al.*, 1998; Thanni, 2006), similar to recent data from GNB bacteraemia in SCD patients (14–26%) (Williams *et al.*, 2009). Salmonella was not a common cause of OM in our study, but MDR-GNB was frequent (62·5%). We know that MDR-GNB colonisation is rising, suggesting that intestinal colonisation might have led to infection in our SCD patients.

Treatment is generally continued for six weeks, but much longer for complicated, MDR infections (Chambers *et al.*, 2000). However, most studies are old and it is likely that for uncomplicated, non-MDR infections, we could use shorter intravenous courses.

Complications are higher than reported in healthy children. Epps *et al.* (1991) reported joint stiffness in 20%, pathological fracture in 6·6% and chronic OM in 27%, which healed in 6–14 months. We had a higher progression to chronic OM (40%) and functional limitation (33%): multifocal, MDR infections probably account for these differences.

Our study has limitations, being a small and heterogeneous sample, but all patients were cared for by the same multidisciplinary team. OAI are still a major health problem in SCD patients and multifocal, severe MDR-GNB infections are emerging, requiring multiple surgical procedures and prolonged antibiotic courses.

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Test trial of spike-in immunoglobulin heavy-chain (*IGH*) controls for next generation sequencing quantification of minimal residual disease in acute lymphoblastic leukaemia

Minimal residual disease (MRD) is the strongest prognostic factor for acute lymphoblastic leukaemia (ALL). Next generation sequencing (NGS) of immunoglobulin/T-cell receptor (Ig/TCR) repertoires is a promising technology for MRD assessment, overcoming many limitations of current quantitative polymerase chain reaction (qPCR) methods. One challenge for using NGS for MRD consists of normalising the number of reads related to the leukaemia clonotype into its molecule copy number (Knecht *et al.*, 2019). In the present study, we validated in a clinical setting the use of immunoglobulin heavy-chain (*IGH*) spike-in control plasmids ($n = 19$) for assessing MRD by NGS (Fig 1A). Repertoire analysis was performed by Vidjil-algo (Duez *et al*, 2016), and MRD calculations were done using an in-house Python script. We achieved results that were better or comparable to those demonstrated by other published methods, which either did not use spike-in controls (Kotrova *et al*, 2015; Shin *et al*, 2017), relied on trade secret methodologies (Faham *et al*, 2012; Cheng *et al*, 2018), or did not explore how their systems work (Ladetto *et al*, 2014; Sekiya *et al*, 2017; Theunissen *et al*, 2019).

In pilot tests, we observed the need of plasmid linearization before use (Figure S1). Additionally, use of spike-ins at high copy numbers (360–1080) consumed a large portion (55·2%) of the sample's reads (Figure S2). Therefore, subsequent experiments were performed using 19 *IGH* V(D)J sequences, comprehending 2–3 different clones per VH segment (VH1–7), at final amounts of 10, 40 and 160 copies per reaction. Detailed methods are available as Supplemental Data.

This spike-in system was validated in retrospective samples from 110 consecutive patients with B-cell precursor (BCP)-ALL (ethical approval Certificado de Apresentação para Apreciação Ética [CAAE]: 57280616.4.0000.5376) that presented *IGH* complete rearrangements, as determined in prospective Ig/TCR screening for MRD analysis by qPCR. The numbers of VH-(DH)-JH clonotypes identified at diagnosis (day 0, D0) by conventional MRD screening and NGS are shown in Table I. NGS identified two or more leukaemia clonotypes in more patients (60%) than homo/heteroduplex screening used in the qPCR method (36·4%). Of note, 20 patients presented with two or more leukaemia clonotypes with identical (IgHD)-N-IgHJ regions, indicating clonal evolution.

The average number of NGS reads/patient in day 35 (D35) follow-up samples was 663 034 (43·8% spike-in). The MRDspike.py script (Data S1) was developed to calculate MRD values. Two fitting models (examples in Fig 1B) for spike-in normalisation were used:

1 *Family fitting:* calculates different predictive models for each VH family, which is applied for each leukaemia clonotype according to its family.
2 *Universal fitting:* a consensus predictive model, independent of VH families, used over Family fitting if: (i) the leukaemia clonotype's VH family cannot be determined; (ii) less than two different spike-in are identified for the leukaemia's VH family; (iii) $R^2 \leq 0{\cdot}8$ for Family fitting.

A total of 129 *IGH* markers had MRD calculated by both qPCR and NGS (Table SIII). Read frequency normalisation into NGS MRD was performed by Family fitting in most cases (86·8%). Read frequencies of spike-in molecules differed across different VH families, likely reflecting variable primer efficiencies (Figure S4). However, MRD by Universal and Family fitting were highly correlated (Figure S5) and equally accurate in comparison to qPCR (Table SIV). Notably, NGS MRD accuracy did not correlate with the number of spike-in reads (Figure S6).